Institut des Nanotechnologies de Lyon

Funded PhD subject
October 2022 – September 2025

Fluidic microsystem for sorting and biophysical characterization of spores of plant pathogenic fungi: the BOTRYPATH project

Educational institution: Université Claude Bernard – Lyon 1 – Ecole doctorale EEA

Location:
Institut des Nanotechnologies de Lyon (INL, https://inl.cnrs.fr/)
Doua Campus, Lyon Villeurbanne – France

Collaborations:
Unité de Microbiologie, Adaptation et Pathogénie (MAP, https://map.insa-lyon.fr/)
Doua Campus, Lyon Villeurbanne – France
CONIPHY (https://conidia.fr/expertises-plateformes/phytopathologie/)
Lyon area

Supervisors:
Jean-François Chateaux (Associate Professor, INL) – jean-francois.chateaux@univ-lyon1.fr
Magalie Faivre (CNRS junior researcher, INL) – magalie.faivre@univ-lyon1.fr
Christophe BRUEL (Professor, MAP) – christophe.bruel@univ-lyon1.fr**Key words:** Lab-On-Chip, fungal spores, electrical impedance spectroscopy, high throughput cell sorting, cell biomechanics.

Application: *A resume and a motivation letter must be sent by email to the 3 supervisors*

Scientif context:

If the context of a growing human population, plant pathogenic fungi are currently considered as a threat to the world's food security, due to losses and damages they cause to crops and harvests. Furthermore, legislation on fungicide use is becoming increasingly stringent in some parts of the World such as Europe. In consequence, a better knowledge of the infectious processes of these micro-organisms appears to be one of the elements that will allow us to respond as well as possible to the challenges of tomorrow in this field. *Botrytis cinerea*, the agent of grey mold, is able of infecting more than 1400 plants, including several of economic importance (grapevine, tomato, strawberry, etc...) [(1)]. It is a model fungus in microbiology because its infectious cycle is controlled in the laboratory, its genome is fully known and many tools have been developed to study it at the molecular level [(2,3)]. These characteristics have strongly contributed to the development of the knowledge acquired on this pathogen [(4)], but technological barriers remain and many aspects of its biology are still unknown.

Objectives of the PhD:

This PhD aims to develop new microfluidic tools (lab-on-a-chip) allowing new manipulations and characterizations of this pathogen:

1) In order to identify the virulence factors of this pathogen, academic laboratories produce genetically modified strains. Due to the large number of nuclei in the cells of this fungus, the last step of the current process requires a long and tedious sorting of the single-nucleate mutant spores. A passive (deterministic lateral displacement) or active (dielectrophoresis) microfluidic pre-sorting will reduce the number of unwanted spores before proceeding to a finer sorting, based on the probing of the number of nuclei in each spore by high-frequency electrical impedance spectroscopy (Coulter-type approach).

2) The interaction of spores with the host plant begins at the interface between the spore wall and the host wall. The biophysical characterization of the fungal wall of wild-type and mutant strains, unexplored to date, will provide new and valuable knowledge on the importance of wall rigidity in the infectious process. This characterization constitutes the second part of this thesis and will be conducted using two complementary approaches mastered in the laboratory:

- an approach based on the response to a mechanical stress on the spore envelop. The mechanical stress can be applied i) by passive micro-fluidics, where only the geometry of the channels and the associated flow will be used to stress the objects to be characterized and/or ii) by transient electro-deformation assays under the action of an electric field. This will result in the first mechanical model of the spore wall of *B. cinerea*.

- an approach based again on impedance spectroscopy, but at lower frequencies this time. This will result in the first electrical model of the spore wall of the pathogen.

By analyzing a dozen wall from mutants and field-collected strains resistant to antifungal agents, and by comparing with data obtained on the wild-type strain, the study will lead to a correlation between the mutated genes, the level of virulence or resistance of the strains and the mechanical and/or electrical profile of the wall. The discovery of specific signatures will open the door to possible new approaches to fungal control and crop protection.

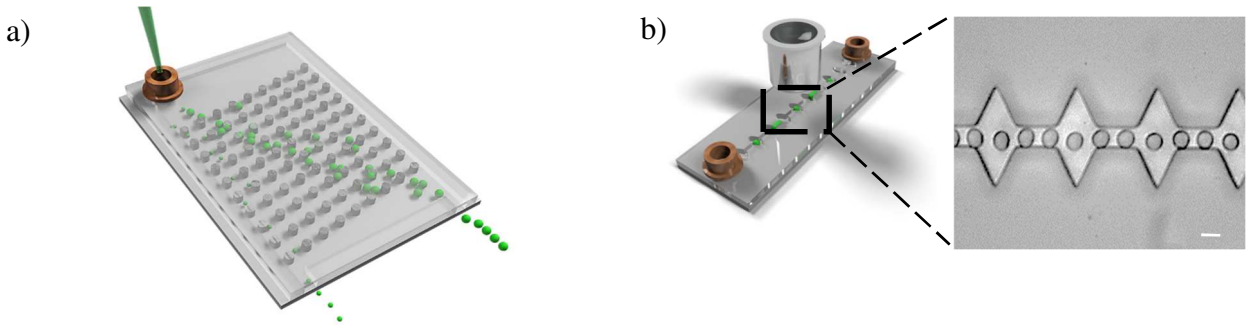

Figure 1: a) Illustration of the Deterministic Lateral Flow based sorting. b) Illustration of passive microfluidic system inducing mechanical stress. The inset corresponds to typical sequence of deformation of a *Botrytis cinerea* spore flowing experimentally in the microsystem.

Original results expected:

Two important advances will be made by this thesis work. First, the development of specific microfluidic tools compatible with high throughput for fungal spores sorting, which is a key parameter in research using mutant strains of fungi. Secondly, these tools will allow the acquisition of new fundamental data on *B. cinerea* such as electrical and mechanical characteristics of its spore's envelop, elements playing a major role in the dispersion of the pathogen and in the early stage of infections (adhesion to the host surface).

Scientific supervision:

The people involved in the day-to-day supervision of the student are:

- Christophe BRUEL (MAP/GFCP): biologist, he is the expert in molecular microbiology of *B. cinerea*. He will also be the end-user of the developed microsystems. As such, he will be involved in the definition of micro-fluidic functions and in their validation.
- Jean-François CHATEAUX (INL/DSE): he will be responsible for the characterization of spores by impedance spectroscopy in an integrated system and for the sorting function of spores by Coulter system.
- Magalie FAIVRE (INL/DSE): she will be in charge of the mechanical characterization of spore membranes by AFM and in micro-fluidic systems.

Thesis funding and environment:

This thesis subject will take place within the framework of the BOTRYPATH project funded by the Rhône-Alpes region. The specific equipment for this project (in particular the impedance spectroscopy system) has already been purchased. The running costs and travels will be covered by the project.

The BOTRYPATH project involves two academic laboratories (INL and MAP) and a company in the Lyon area (CONIDIA - CONIPHY) specialized in fungal diseases of plants.

The student will benefit from the technological infrastructures of the INL (350 m² of clean room + 65 m² of laboratory L2) and, if necessary, from the infrastructures of the laboratory of the Génétique Fonctionnelle des Champignons Phytopathogènes team.

Profile of the desired candidate:

Ideally, the candidate has a background as a biophysicist with a first experience in the field of lab-on-chip dedicated to cell manipulation. However, the position is more widely open to anyone interested in the field and having skills in (bio)mechanics, (bio)impedance, instrumentation or microfluidics... This subject has a strong experimental component.

Skills developed during the PhD:

This thesis will allow the candidate to acquire a broad multidisciplinary expertise in the following domains:
(i) Microfluidics: design of microfluidic chips (CLEWIN...), clean room fabrication (soft lithography, polymer replication...) and use with mushroom spores.
(ii) Impedance: electrode design, clean room fabrication, measurement (lock-in amplifier), programming and signal processing.
(iii) Biophysics: in-depth knowledge of cell mechanics and image analysis (Matlab®),
(iv) Biological: culture of microscopic fungi, recovery of spores.

Professional prospects after the PhD:

This subject is a perfect starting point for an academic career. The multidisciplinary character of the thesis is a guarantee not only of scientific quality, but also of a stimulating scientific environment. The valorization of this subject will be done through publications in scientific journals with high impact factor, and participation in international conferences.
As far as a career in industry is concerned, the acquired skills can be easily valorized. In particular, experimental, characterization and nanotechnology skills are highly valued in the microelectronics or medical diagnostics industries. Moreover, the involvement of the company CONIDIA - CONIPHY, could be a plus for the integration of the student in the local socio-economic world.

Bibliography about the PhD subject:

Botrytis Cinerea:
1. S. Fillinger and Y. Elad. "*Botrytis* - The Fungus, the Pathogen and its Management in Agricultural Systems, Springer, (2016).

2. J. Schumacher. "Tools for *Botrytis cinerea*: New expression vectors make the gray mold fungus more accessible to cell biology approaches". Fungal Genetics and Biology, 49(6): 483-497, (2012).

3. L. Castillo, V. Plaza, L.F. Larrondo and P. Canessa. "Recent Advances in the Study of the Plant Pathogenic Fungus Botrytis cinerea and its Interaction with the Environment". Curr Protein Pept Sci., 18(10):976-989, (2017).

4. A. de Vallée, P. Bally, **C. Bruel**, L. Chandat, M. Choquer, C. Dieryckx, J.W. Dupuy, S. Kaiser, M.P. Latorse, E. Loisel, G. Mey, G. Morgant, C. Rascle, J. Schumacher, A. Simon, E. Souibgui, M. Viaud, F. Villalba, N. Poussereau. "A Similar Secretome Disturbance as a Hallmark of Non-pathogenic Botrytis cinerea ATMT-Mutants?". Front Microbiol. Dec 6;10:2829, (2019).

Microsystem for the impedance spectroscopy:
J.A. Martinez Santa Maria, A. Montalibet, E. McAdams, **M. Faivre** and R. Ferrigno, "Effect of electrode material on the sensitivity of interdigitated electrodes used for Electrical Cell-Substrate Impedance Sensing technology", Conf. Proc. IEEE Eng. Med. Biol. Soc., 813-816, (2017).

J.A. Martinez SantaMaria, A. Montalibet, E. McAdams, **M. Faivre** and R. Ferrigno, "Comparison of ITO and IrOx-modified ITO interdigitated electrodes for Electrical Cell substrate Impedance Sensing (ECIS) applications", Proceedings, 1(4):532, (2017).

Microsystem for the cellular deformability assays:
A. Amirouche, R. Ferrigno and **M. Faivre**, "Impact of channel geometry on the discrimination of mechanically impaired Red Blood Cells in passive microfluidics", Proceedings, 1(4):532, (2017).

A. Amirouche, **M. Faivre**, **J.F. Chateaux** and R. Ferrigno (2017) "Determination of Red Blood Cell fatigue using electrodeformation", Conf. Proc. IEEE Eng. Med. Biol. Soc., 3584-3587, (2017).